ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS)

Significant progress has been made since 1981, when mysterious cases of pneumonia led researchers to identify the disease now known as AIDS. Research has led to a better understanding of the structure of human immunodeficiency virus (HIV), which causes AIDS, how HIV attacks the immune system, the role of the immune system in controlling HIV infection, and how to intervene therapeutically. Potent therapeutic regimens, commonly referred to as highly active antiretroviral therapy, or HAART, have been successful in suppressing HIV to virtually undetectable levels in the blood and decreasing the incidence of opportunistic infections. HAART has greatly improved the quality of life of many HIV-infected people worldwide and has led to a dramatic decline in AIDS-related deaths.

Despite these scientific advances, the HIV/AIDS pandemic continues to rage around the world, with an estimated 40.3 million people living with the disease. In 2005, 3.1 million people died from AIDS, and 4.9 million people were newly infected with HIV. Of the 4.9 million new infections, 700,000 were in children. Globally, just under half of all people living with HIV are female. An estimated 40,000 people have been infected with HIV each year in the United States in the past 10 years, but the epidemic is now disproportionately lodged among African Americans and is affecting much greater numbers of women.

NIAID research fellow Dr. Johnan Kaleeba, of the Laboratory of Molecular Microbiology in the Division of Intramural Research, and an assistant study HIV gene regulation.

Since the beginning of the epidemic, NIAID's comprehensive research program has been at the forefront in the fight against HIV/AIDS. NIAID supports a broad array of domestic and international HIV/AIDS research programs and collaborates with more than 40 countries through investigator-initiated research grants and multicenter prevention, vaccine, and therapeutic research networks. (See Division of AIDS Overview on page 15 for a description of programs.) With a growing number of research programs and initiatives, NIAID is poised to tackle new global research challenges as well as the changing demographics of the HIV/AIDS epidemic.

Basic Research

Basic research in HIV pathogenesis, microbiology, immunology, virology, and animal model development lays the foundation for advancing research in HIV treatment and prevention. At NIAID, this research is conducted primarily through investigator-initiated research as well as a number of targeted programs and several large cohort studies.

This past year, as a result of advances in basic science, researchers have learned that HIV exploits a large residential population of resting memory cells in the gut-associated lymphoid tissue (GALT) to produce high amounts of virus and deplete critical memory CD4+ T cells, thereby initiating the disease process that leads ultimately to AIDS.

Several human genes have been identified as being essential for HIV infection. The most notable is the CCR5 gene, which codes for a chemokine receptor that also acts as an HIV co-receptor on the surface of susceptible

cells. Individuals with a deletion of one or both copies of the CCR5 gene are less likely to become infected with HIV. Another gene related to HIV susceptibility is CCL3L1, which encodes a protein that binds to CCR5 and blocks HIV entry into host cells. In 2005, a team of NIAID-supported scientists discovered a new twist to the story; it is not just the presence or absence of the CCL3L1 gene, but also the number of gene copies that can affect susceptibility to HIV infection and severity of HIV disease. NIAID intramural investigators added another surprise: working with a mouse model, they discovered that the CCR5 co-receptor needed for initial HIV infection and for subsequent disease progression also functions to control West Nile virus disease by helping to clear virus from the brain. This has important implications for the development of HIV inhibitors that act by blocking CCR5, because such agents, which could be helpful in treating HIV, might also render people more susceptible to the brain inflammation that can result from West Nile virus infection.[1]

Several investigators have recently identified a pair of gene families that encode proteins with antiviral effects. These genes are referred to as the APOBEC and tripartite motif (TRIM) families. Both gene families have multiple members. APOBEC has close to 30 and TRIM has close to 70, with the functions of all the family members not yet defined. APOBEC3G (A3G) and APOBEC3F (A3F) proteins have been shown to cause lethal mutations in HIV and other retroviruses. HIV counters the effects of these proteins through its Vif protein, which promotes degradation of A3G and A3F. Recent work by an NIAID-funded investigator has unveiled a second antiviral function of A3G that protects resting CD4+ T cells against HIV infection.

The TRIM gene family was first defined by the identification of TRIM5alpha as a factor from Rhesus macaques that had strong anti-HIV activity. Since this initial discovery, the number of genes identified in the TRIM family has grown, and five members have been shown to have anti-HIV activity. TRIM1 and 5 work in the cytoplasm, TRIM19 and 23 appear to work in the nucleus at the time of integration, and TRIM32 works by interfering with HIV Tat. What remains to be defined is how HIV successfully mitigates the effects of these antiviral factors. Taken together, these gene products constitute a new arm of the innate immune system, the intracellular innate immune system.

NIAID scientists at the Vaccine Research Center (VRC) used a simian immunodeficiency disease (SIV) model of HIV infection to investigate the role of the virus in the depletion of memory CD4+ T cells during the acute phase of infection. Using a technique that can detect very low amounts of virus in cells, these scientists demonstrated that acute SIV infection is associated with very high rates of infection and the subsequent depletion of memory CD4+ T cells not only in mucosal tissue, but also in peripheral lymphoid tissue and blood. In fact, they found that 30 to 60 percent of CD4+ memory T cells throughout the body were infected by the virus at peak infection. In the first four days following peak infection, 80 percent of these cells were destroyed by the body, corresponding to a remarkable 24 to 48 percent elimination of *all* of the memory CD4+ T cells in this short timeframe.

The results from this study indicate that the loss of memory CD4+ T cells during acute SIV infection is considerably more marked than previously thought. The extent of the massive and rapid loss of memory CD4+ T cells throughout the body—not just in mucosal tissue where the virus typically enters the host—during acute SIV infection has critical implications for HIV vaccine development and interventional therapies. These findings indicate that preventive and therapeutic strategies must be designed to prevent early and massive destruction of the memory CD4+ T cells

by reducing viral load during the acute phase of infection.

NIAID scientists made other important basic research discoveries in FY 2005. For example, they discovered a human enzyme that is crucial to HIV replication. The process of how HIV genetic material—a long unedited strand of RNA—exits the host cell nucleus has long puzzled scientists. Human cells cut, edit, and splice RNA before it can leave the nucleus, but somehow HIV subverts that process and exports the long version of RNA that encodes instructions for making new viral particles. The researchers found that HIV uses a human enzyme known as DDX3 to straighten its RNA before threading it through small pores in the nuclear membrane. This work offers the first evidence that HIV must use this human enzyme to replicate, and suggests a potential new target for drug development.[2]

Intramural researchers also expanded their basic knowledge of the cytokine, interleukin-2 (IL-2), and how it functions. Intermittent administration of IL-2 to HIV-infected individuals has been found to increase the number of certain kinds of CD4+ T cells. NIAID scientists used sophisticated lab tests to track over time the fate of CD4+ T cells produced during IL-2 therapy of HIV-infected individuals. Their experiments revealed that the CD4+ T cell expansions were the result of prolonged CD4+ T cell survival, which affected both naïve and central memory CD4+ T cells without significantly affecting CD8+ T cells. These findings suggest that IL-2 can help maintain cells important for host defense against new antigens and those needed for long-term memory to opportunistic pathogens. They also demonstrate the back-and-forth nature of translational research. Years of basic lab research on IL-2 led to these patient trials, which are now increasing our basic understanding of IL-2 functioning in immune cells. This knowledge will, in turn, provide a greater foundation for understanding clinical observations in ongoing patient trials of IL-2.[3]

Although much has been learned, questions still remain about (1) the mechanisms of viral entry into and exit out of host cells, including the roles of cellular co-receptors and other host cell molecules; (2) the structure, function, and mechanism of action of viral genes and proteins, and how they interact with host cell genes and proteins to affect HIV replication; (3) host factors that modulate HIV transmission, replication, establishment of infection, and disease progression; and (4) the elements of the immune response to HIV during primary and chronic infection and their roles in controlling disease establishment and progression. Answering basic scientific questions about how the virus attacks the body and how the body defends itself is critical to providing additional potential targets against which therapeutic interventions and vaccines can be directed.

Vaccine Research

An HIV vaccine that is simple to administer, inexpensive, and induces long-lasting immunity against all HIV subtypes is critical to the effective control of the global spread of HIV. It is, therefore, one of NIAID's highest priorities, albeit one of the most difficult challenges in HIV/AIDS research. NIAID supports a spectrum of HIV vaccine research and development activities, including basic research (discovery), preclinical screening and animal model development, product development and manufacturing, and clinical research. The scope and breadth of these programs and resources continue to significantly advance global HIV vaccine development efforts.

Over the years, NIAID-supported HIV vaccine research has led to the identification of new and innovative HIV vaccine designs, improvements in vaccine delivery, development of innovative laboratory techniques and animal models for evaluating vaccines, and evaluation of over 50 vaccine candidates in clinical studies. Additional studies have already been initiated or are being planned to evaluate the safety and

immunogenicity of a range of new candidate vaccines, including lipopeptide vaccines alone and in combination with a canary pox vaccine, a Venezuelan equine encephalitis replicon vector vaccine, novel DNA vaccines, a recombinant nonreplicating adenovirus vaccine, modified vaccinia Ankara and other novel pox vector-based vaccines, a cytotoxic T lymphocytes multi-epitope peptide vaccine, and molecular adjuvants.

Notably, a NIAID-funded study provided an explanation why it is difficult to induce broadly reactive neutralizing antibodies against HIV-1. Many strains of HIV circulate worldwide, and designing HIV vaccines against the viral envelope capable of eliciting antibodies that neutralize these different strains continues to represent a major research challenge. Despite significant advances in our understanding of HIV/SIV envelope structure and function, no envelope-based vaccine has thus far succeeded in eliciting antibodies that match the breadth of the few, broadly neutralizing human monoclonal antibodies derived from long-term HIV-infected individuals. In a recent study, the authors found that not only do these human antibodies react and neutralize HIV, but they also react to cardiolipin, a lipid molecule component first isolated from heart muscle and subsequently found in the membranes of mitochondria of all cells. The implication is that these rare, broadly neutralizing antibodies might have been isolated from individuals with immune dysfunctions in whom the body makes antibodies against their own tissue molecules. This study raises many questions regarding the ontogeny of broadly neutralizing anti-HIV antibodies and the potential to elicit this type of antibody response in normal individuals.

In its fifth year, the HIV Vaccine Trials Network (HVTN) continued to make improvements towards its operations and comprehensive goals. These include (1) streamlining protocol development to shorten the time needed to initiate new studies; (2) strengthening the HVTN Clinical Coordinators Working Group, which assists and prepares new units for clinical research; (3) developing analytical criteria to evaluate the suitability of budgets submitted by units; (4) promoting junior investigators for leadership roles through selection to chair/cochair positions on protocols or HVTN committees; (5) continued strengthening of laboratory programs through expansion of proficiency testing and assay validation; (6) conducting more trials at participating international sites than in previous years; and (7) helping international sites develop plans for access to antiretroviral therapy for volunteers who acquire HIV infection during trial participation.

NIAID scientists at the VRC examined the breadth of T cell repertoire, i.e., the number of different T cells that make up the response to the AIDS virus. These researchers showed that T cell responses that are narrow in their repertoire cannot tolerate viral mutations and allow the virus to escape rapidly. Conversely, T cell responses that have a broad repertoire are likely more able to tolerate mutations and thus can contain the virus more easily. These considerations are extremely important in helping to establish a framework on which to base rational design of HIV vaccines.

(See the "Vaccine Research and Development" section on page 138 for additional vaccine information.)

Nonvaccine Prevention Research

To control the HIV/AIDS pandemic, new and more effective methods and strategies are needed to prevent HIV infection. Until a highly efficacious vaccine is developed, control of the pandemic will still require a combination of prevention approaches. NIAID's HIV Prevention Trials Network (HPTN) develops and tests promising nonvaccine strategies to prevent the spread of HIV/AIDS, including:

- Drugs or vaccines that are practical and easy to use to prevent mother-to-child

transmission (MTCT) of HIV, including prevention of viral transmission during breastfeeding;

- Microbicides to prevent sexual transmission of HIV;
- Antiretroviral therapy (ART) that could reduce the spread of HIV from infected persons to their sexual partners;
- Measures to control other sexually transmitted diseases and thereby decrease the risk of co-infection with HIV;
- Interventions to reduce behavior that exposes people to HIV; and
- Interventions to curb the spread of HIV among those who use intravenous and non-injection drugs.

NIAID-funded research within the HPTN has led to important scientific advances that increase our understanding about the transmission of HIV. These findings provide a foundation for developing and testing innovative prevention strategies.

Notably, microbicide research funded by the Division of AIDS has shown that combination microbicides can result in what appears to be synergistic inhibition of virus transmission in monkeys. Additional studies using a chemically modified form of RANTES (PSC-RANTES) have successfully provided the first proof that disruption of the virus–co-receptor interaction can afford complete protection of monkeys vaginally exposed to simian-human immunodeficiency virus (SHIV). Prevention research involving topical microbicides is described in the "Sexually Transmitted Infections" section on page 126.

Therapeutics

One of the primary goals of HIV/AIDS therapeutic research is to evaluate new treatments and innovative treatment strategies for HIV/AIDS and HIV-associated complications and infections. As a result of HAART, the life expectancy of HIV-infected individuals has dramatically increased. As individuals live longer with HIV, there is a greater likelihood of the development of drug resistance, metabolic abnormalities, co-infections, and drug toxicities. Moreover, the immune system only partially recovers during HAART treatment. Thus, new therapies and ways to expand the clinical benefit of currently approved therapies are still urgently needed. NIAID's therapeutics research programs support research to address these issues.

In addition to a comprehensive clinical research agenda in the Unites States, NIAID fosters the study of HIV and HIV-associated infections internationally, including research in resource-poor countries. Key issues to be addressed in these countries include therapeutic regimens suitable for resource-poor settings; when to start HIV therapy; how to monitor safety and efficacy with minimal laboratory resources; interactions of endemic infections and HIV; strategies to prevent and treat co-infections; and drug interactions, including the drugs used to treat endemic infections. Efforts are underway to provide training to healthcare workers in developing countries to improve the management and treatment of individuals infected with HIV, as well as training for U.S. researchers in the healthcare needs of resource-poor countries. This is being accomplished through a variety of mechanisms, including the expansion of existing clinical trials groups to collaborate with investigators in resource-poor countries, direct funding of investigator-initiated research through R01 awards, and the development of comprehensive HIV research centers through the Comprehensive International Program of Research on AIDS. Through the Small Business Innovation Research mechanism, NIAID continues to support many small businesses seeking to develop technologies for monitoring patients in resource-limited settings, where

standard technologies like CD4+ and viral-load testing might be unaffordable.

The majority of NIAID's therapeutic clinical research is conducted through the clinical trials networks, Adult AIDS Clinical Trials Group (ACTG), the Pediatric AIDS Clinical Trial Group, and the Terry Beirn Community Programs for Clinical Research on AIDS. These networks conduct, at any given time, over 100 clinical studies to address a full range of HIV and HIV-associated infections, and complications. In addition to studies to assess treatments and treatment strategies, the networks conduct research in the following areas: co-infections such as hepatitis C virus (HCV); tuberculosis (TB); metabolic complications of HIV and HAART; opportunistic infections; treatment of naïve patients; salvage therapy, women's health, and the prevention of MTCT of HIV.

Currently, NIAID is conducting a large international clinical trial (ACTG 5175) to evaluate the efficacy of protease inhibitors and non-nucleoside reverse transcriptase inhibitors containing therapy combinations for initial treatment of HIV-infected individuals from diverse areas of the world. ACTG 5175 has completed stage 1 enrollment of patients in the following international sites: Johannesburg and Durban, South Africa; Chiang Mai, Thailand; Lilongwe and Harare, Malawi; and Lima, Peru. In addition, NIAID continues to support two large multicenter studies, the Strategies for Management of Anti-Retroviral Therapy (SMART) study (*www.smart-trial.org*) and the Evaluation of Subcutaneous Proleukin in a Randomized Interventions (ESPRIT) study (*www.niaid.nih.gov/dir/labs/lir/hiv/esprit.htm*).

In addition to the primary treatment studies, NIAID also supports ongoing network studies to address the timing and selection of antiretroviral regimens in the setting of acute opportunistic infections and co-infections such as TB and HCV, as well as treatment strategies to prevent and treat metabolic disorders. One such study is a phase II long-term maintenance therapy trial designed to study whether long-term maintenance with pegylated-interferon (PEG-IFN) reduces the rate of disease progression in subjects with HCV/HIV co-infection who did not respond to the standard treatment regimen of PEG-IFN plus ribavirin (ACTG 5178).

The Maintaining Options for Mothers Study is a prospective, randomized clinical trial to evaluate the effectiveness of three different antiretroviral regimens for the prevention of nevirapine (NVP) resistance after single-dose NVP has been administered during delivery. Another important study, Optimal Combined Therapy after NVP Exposure is a phase III trial that compares the response of two different classes of antiretroviral drugs in women who have received only a single dose of NVP (ACTG 5208, OCTANE).

NIAID also continues to evaluate new classes of antiretroviral compounds, including viral entry inhibitors, which show increasing promise in preclinical and clinical studies. Building on the success of the fusion inhibitor, Fuzeon, NIAID is conducting studies focused on developing an orally available drug that will fight HIV at the point of entry.

NIAID Intramural Research

Although recent advances in the treatment and monitoring of HIV-1 infection have substantially diminished HIV-associated illness and mortality, the management of HIV-infected patients has become increasingly complex. Both the acute and long-term toxicities of the common antiretroviral medications are becoming better understood and continue to complicate the successful management of this condition. Alternative treatment strategies are clearly needed, both for patients with access to ART and for the much greater number of patients with little or no access to therapy. NIAID clinical research is addressing many issues and strategies to improve patient care, including:

- Optimal dosing of anti-retroviral drugs;
- Integration of immune-based therapies with ART;
- Development of a successful ART strategy that employs periodic treatment interruptions;
- Optimal use of immune-based therapies to decrease exposure to ART;
- Characterization of immune recovery in persons with advanced HIV infection and discontinuation of prophylaxis against opportunistic infections; and
- Management of ART in individuals with advanced infection.

Studies employing serial interruptions of HAART seek to determine whether periodic treatment interruptions might offer therapeutic equivalence to continuous therapy.

The potential to substantially reduce the amount of medication required to effectively treat HIV infection could have widespread implications for the global effort to contain and treat the disease in terms of financial cost, toxicity to patients, and adherence to therapeutic regimens. In addition, periodic interruptions in ART could offer insights into the pathogenesis of HIV infection, immune responses, and other factors important to an individual's ability to control HIV replication.

Another major area of NIAID intramural research focuses on characterizing the immunologic abnormalities associated with HIV infection, developing immunologic approaches to the therapy of patients with HIV infection, and utilizing these immune-based therapies as tools for obtaining additional insights into the pathophysiologic mechanisms present in patients with HIV infection.

Specifically, this research aims to reverse the CD4+ cell decline associated with progressive HIV-1 infection through the use of subcutaneous administration of IL-2. A series of randomized phase I/II studies have established this as a feasible method for increasing the CD4+ count in patients with HIV infection. These studies were then extended to optimize the dosing regimens for maximal immunologic and virologic benefit while minimizing side effects. Cohorts of patients are being followed who have received IL-2 treatment for periods that now extend to almost 10 years. Collaborations with a large number of extramural colleagues, both in the United States and abroad, aim to determine whether the favorable effects of IL-2 therapy on CD4+ counts translate into a significant delay in the onset of AIDS-defining conditions and/or death in recipients of IL-2 plus ART versus patients on ART alone.

NIAID researchers have also performed phase I/II studies with a novel CCR5 inhibitor compound and are conducting phase II efforts to better characterize the activity, pharmacokinetics, and efficacy of this agent, both alone and when added to a conventional HAART regimen.

Efforts are continuing to improve access to clinical trials for local minority populations through an outreach program that includes a close relationship with local clinics for the medically underserved. Finally, NIAID researchers have played an active role in helping establish a clinical research infrastructure in the South African National Defense Force military healthcare system through participation in the HIV research projects organized under Project Phidisa.